Exposure of cells to carcinogenic nickel compounds induces many genes that are commonly expressed in cancer cells but not in normal cells.

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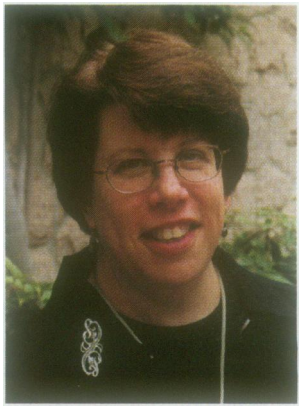

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Nickel Carcinogenesis, Mutation, Epigenetics, or Selection

Nickel compounds have been well established as human carcinogens. Investigations into the molecular mechanisms of nickel carcinogenesis have revealed that not all nickel compounds are equally carcinogenic: certain water-insoluble nickel compounds exhibit potent carcinogenic activity, whereas highly water-soluble nickel compounds exhibit less potency (*1*). The reason for the high carcinogenic activity of certain water-insoluble nickel compounds relates to their bioavailability and the ability of the nickel ions to enter cells and reach chromatin. The water-insoluble nickel compounds enter cells quite efficiently via phagocytic processes, and subsequent intracellular dissolution yields very high cellular levels of Ni^{2+}. Mathematical estimations indicated that if a 1.45-μm nickel sulfide particle totally dissolved in a cell, the potential nickel concentration would be 250 mM, and if a 4.0-μm nickel sulfide particle totally dissolved in a cell, the potential nickel concentration would be 4.75 M. Thus, the process of phagocytosis represents a very efficient manner for the accumulation of nickel inside the cell.

Nickel is classified as a borderline metal ion because it has both soft and hard metal properties and it can bind to sulfur, nitrogen, and oxygen groups. Nickel ions are very similar in structure and coordination properties to magnesium. Like magnesium, nickel binds to the oxygen of the DNA phosphate backbone; but like copper and cobalt, nickel enjoys a high affinity for the imidazole nitrogen of histidine in proteins.

Investigations of the mutagenic activity of highly carcinogenic nickel compounds have failed to reveal much activity in most of the mutational systems examined thus far (*2*). However, carcinogenic nickel compounds induce chromosomal aberrations, including those that are specific to heterochromatic chromosome regions (*3*). Carcinogenic nickel compounds have also been shown to increase the extent of DNA methylation. In one experimental system in which transgenes were located either near or distant from a heterochromatic chromosome region in hamster cells, carcinogenic nickel compounds were shown to hypermethylate the transgene located near heterochromatin, but not the transgene more distant from heterochromatin (*4*). Although the mechanisms by which nickel induces DNA hypermethylation are presently unknown, a possible model might include the ability of nickel to substitute for magnesium and increase DNA and chromatin condensation more efficiently than magnesium, thereby triggering a de novo DNA methylation of the genome region that was condensed by the presence of nickel (*5*). Other genes such as tumor-suppressor and senescence genes that are near this heterochromatin region may also become methylated (*6,7*). Thus, the ability of nickel to induce DNA hypermethylation is an attractive mechanism by which nickel may reprogram a normal cell into a cancer cell without inducing mutations by causing the loss of expression of tumor-suppressor genes by de novo DNA methylation of their promoters. Calculations performed on nickel-induced transgene inactivation by DNA methylation indicated that this incidence could be as great as 1 in 1,000, as compared to mutagenic events that would occur at the rate of 1 in 100,000 to 1 in 1,000,000 (*4*).

In a related line of experiments, we recently examined the effects of nickel on gene silencing in yeast using a marker gene placed near a telomere binding element (*8*). As with the mammalian systems, we found that nickel was capable of silencing genes placed within a certain proximal distance of a yeast telomere-silencing element. As the distance between the target gene and the silencing element increased, the effect of nickel on silencing the marker gene was abolished. Further studies have revealed that nickel depressed the acetylation of histone H4 in the N- terminal tail of this histone, and the loss of lysine acetylation was confined to lysines surrounding a nickel-anchoring site at the histidine 20 residue of histone H4. Additional studies are needed to determine specifically how nickel coordination at this site might decrease histone acetylation that is associated with the loss of gene expression following a nickel-induced increase in chromatin condensation. Although yeasts do not exhibit mammalian patterns of DNA methylation of cytosines, histone acetylation patterns in yeast are inherited as DNA methylation patterns are inherited in mammalian systems.

In addition to producing mutations, chromosome aberrations, and gene silencing by DNA methylation, exposure of cells to nickel also induces a variety of gene expression changes that yield cells with a spectra of expressed genes similar to cancer cells. For example, nickel is very efficient at turning off the expression of thrombospondin (*9,10*), a gene product that is known to be antiangiogenic because the proliferation of a cancer cell depends on angiogenesis to promote the growth of a blood supply that ensures the flow of nutrients toward the tumor. Thrombospondin is transcriptionally downregulated in nickel-transformed cells (*9*). Additionally, nickel is very efficient at inducing a hypoxia-like state in cells (*11*). Thus, nickel has been found to induce the hypoxia-inducible factor (HIF) which is responsible for increasing the expression of glycolytic enzymes and other genes that allow cells to survive under low oxygen tension. The mechanism by which nickel induces this hypoxic state is currently under investigation; however, it is of interest that exposure of cells to carcinogenic nickel compounds induces many genes that are commonly expressed in cancer cells but not in normal cells. Because the induction of genes and changes in DNA methylation are both very frequent events that are readily induced by exposure to nickel compounds, these events are likely to be important factors in the carcinogenesis of nickel compounds, in contrast to the production and

fixation of mutations which are much less frequent events that require substantial cell proliferation.

Not only have nickel compounds been shown to be responsible for a number of human cancers in occupationally exposed workers, but carcinogenic nickel compounds have been shown to induce many different types of tumors in experimental animal systems. For example, carcinogenic nickel compounds have been shown to induce muscle tumors, such as rhabdomyosarcoma, following intramuscular injections of nickel (*1*). Muscle tumors are not common cancers. Thus, the potent ability of carcinogenic nickel compounds to induce a cancerous state may be related to a selective advantage that could be important in the carcinogenic process, particularly if the nickel-exposed cells have preexisting mutations. As discussed above, the cellular balance of expressed genes may be so fragile that nickel can readily upset the status quo. The short-term carcinogen-induced alterations of gene expression may establish a selective advantage for cells that already harbor mutations or other perturbations of DNA repair or cell cycle controls. This concept is particularly interesting in view of the mounting experimental data which suggest that genetic predisposition is very important in the etiology of human cancer. Although our exposure to carcinogens undoubtedly plays an important role in the development of some cancers, genetic predispositions can determine which individuals will ultimately get cancer and which ones will not. Whether additional mutations are at the heart of cancer induction by environmental carcinogens remains a dogma that needs to be challenged by the notion that genetic predisposition may be responsible for the multitude of mutations detected in cancer cells. Thus, the driving force for environmentally induced cancers may not initially be mutations, but may, instead, be related to carcinogen-induced selection through epigenetic changes that eventually establishes a gene expression program characteristic of a cancer cell. As suggested by the growing literature on nickel carcinogenesis, the initial events in environmentally induced cancers may be a combination of gene induction and gene silencing by epigenetic DNA methylation that leads to cancer cell selection. Determination of the extent to which this concept applies to other environmental carcinogens is the challenge of the future.

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